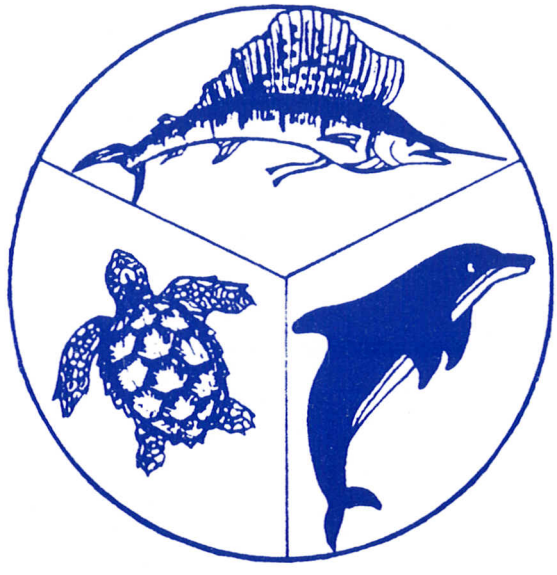
NOAA TECHNICAL MEMORANDUM NMFS SEFC - 277

MARINE FORENSICS PROGRAM:

A BIOCHEMICAL METHOD TO DISTINGUISH WILD FROM CULTURED FISH



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March 1991

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Center
Charleston Laboratory
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A BIOCHEMICAL METHOD TO DISTINGUISH WILD FROM CULTURED FISH

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ABSTRACT

Research is currently underway to develop a biochemical method to distinguish wild (illegally caught) from cultured fish. The objective is to use fatty acid compositional differences to differentiate wild from cultured fish.

Results indicate that in addition to linoleic acid (18:2n6), differences in the concentrations of other long chain polyunsaturated fatty acids such as linolenic acid (18:3n3), arachidonic acid (20:4n6), docosapentaenoic acid (22:5n6), and docosahexaenoic acid (22:6n3) could also be used to help distinguish wild from cultured fish.

INTRODUCTION

Considerable interest is focused on commercially farming several fishes (e.g., striped bass, Morone saxatilis, and hybrid striped bass, white bass, M. chrysops, x striped bass cross), which are now listed as game fish in many states (Parker 1988; Smith 1988). As such, several states have or are considering legal provisions for the commercial growing of these fish (Sharpe and Moore 1987; Parker and Miller 1988). A major concern has been the lack of a biochemical method to distinguish wild (illegally caught) from cultured (farmed) fish.

As part of the National Marine Fisheries Service (NMFS) Charleston Laboratory's Forensic Program, a cooperative agreement was established with the South Carolina Wildlife and Marine Resources Department (SCWMRD) with the goal to develop forensic techniques in areas of mutual interest. In particular, one objective was to develop a biochemical method to distinguish wild from cultured fishes which were considered game species in SC and which were being reared in aquaculture. A second objective was that the method should utilize the edible portion of the fillet. Based on these observations we examined the possibility of using fatty acid composition differences to differentiate wild fish from cultured fish (Jahncke et al. 1988b, 1989; Jahncke and Seaborn 1989).

Joseph et al., (1985) suggested that fatty acids could be used as a tool for positive identification of marine turtle oils in cosmetics. Grahl-Nielsen and Ulvund (1988) noted that fatty acid compositional differences had potential for identifying different populations of herring (Clupea harengus). Additionally, Knutsen et al., (1985) used fatty acid analyses to distinguish cod (Gadus morhua) eggs from haddock (Melanogrammus aeglefinus) eggs.

Fatty acids in wild fish have been shown to reflect the fatty acids characteristic of the food chain (Linko et al. 1985). Wild fish are an excellent source of omega-3 fatty acids as are most plankton species (Ackman 1982). Cultured fish fed a diet based primarily on soybean or other vegetable sources, on the other hand, contained high concentrations of omega-6 fatty acids; especially linoleic acid (18:2n6) (Chanmugam et al. 1986; Jahncke et al. 1988a, 1988b; Jahncke and Seaborn 1989).

Fatty acids in fish vary by season, sex, species, location, diet, physiological condition, etc. (Stansby 1981). Therefore to develop sufficient baseline data for enforcement purposes, fatty acid compositions will be determined for wild striped and wild hybrid striped bass collected from five major South Carolina state waters at three month intervals for two years. The study was initiated in November 1988 and is scheduled for completion in July 1990. Approximately 500 fish have been collected for the first year.

Cultured hybrid striped bass and diet samples have been analyzed over three years to determine their fatty acid composition. Because of the interest in striped bass and hybrid striped bass aquaculture and the need to protect wild stocks, members of the American Fisheries Society Striped Bass Technical Committee have recently agreed to send samples of wild striped bass and wild hybrid striped bass from their states. Several commercial growers have also agreed to provide cultured fish and diet samples for fatty acid analyses. Thus, at completion of this cooperative study sufficient information should be available for use in forensic activities throughout the U.S.

To test the reliability of our methods for distinguishing wild from cultured fish, seven Test Fish Samples were brought to the Charleston Laboratory by SCWMRD Law Enforcement personnel for analysis. The objective was to identify the samples as to whether they came from wild or cultured fish using fatty acid composition differences.

This paper provides data on the fatty acid composition of wild fish collected in November 1988 as well as the results from the "Test Case". Representative fatty acid compositions are also given for cultured hybrid stiped bass.

MATERIALS AND METHODS

Collection of Reference Fish

Wild fish (both striped bass and hybrid striped bass) were collected and identified by SCWMRD Fishery Biologists and provided to the NMFS, Charleston Laboratory for fatty acid analysis. All fish were identified as to species, size, sex, sexual maturity and collection site (Figure 1). The objective was to collect approximately 50-60 fish per site per collection date. Actual numbers of fish collected depended upon season and availability. The number of fish collected ranged from a minimum of five fish (Jan., 89 - Lake Wateree) to a maximum of 52 fish (Jan., 89 - Lake Murray)

General collection procedures were:

1. The specific collection sites were: 1) Santee Cooper system, 2) Lake Wateree, 3) Lake Murray, 4) Lake Thurmond (Clarks Hill) and 5) Lake Hartwell (Figure 2).

2. Samples were collected in November 1988, January, April and July, 1989. All sites were sampled over a maximum period of 30 days.

3. Fish sizes included the range of illegally caught fish sizes (approximately 400-3500g).

4. Fish were iced at the time of collection and then frozen as soon as possible. Small fish were frozen whole. Large fish were sub-sampled (200 g minimum of flesh, skinless preferred) instead of freezing the whole fish.

Sample Preparation of Reference Fish

The reference fish were grouped by location, season, species, size, sex and sexual maturity. Frozen skinless fillets, from individual fish, were homogenized with belly flap, nape and tail section removed. Composite and individual samples were prepared. Composite samples consisted of approximately 5 fish per composite. Using 20 g from each individual fish homogenate, a single 100 g composite was formed. One hundred gram samples were also prepared from individual fish to ensure that the range of fish sizes, sex, sexual maturity, species and sites were adequately sampled.

Receipt and Preparation of Law Enforcement Test Samples

Seven groups of unfrozen and unidentified skinless fillets labeled A, B, C, D, E, F and G were delivered to the Charleston Laboratory by a Law Enforcement Official. The fresh samples were immediately iced and placed in a cold storage room (+3°C).

The size and weight of the seven test samples varied. Sample C was the smallest, consisting of two fillets weighing a total of 13 g. Sample G was the largest, consisting of several fillets weighing a total of 450 g. The five remaining samples (A, B, D, E, and F) each contained two fillets (approximately 110 g in total weight).

The skinless fillets, with belly flaps removed, were rinsed with tap water before preparation. One fillet from each sample was homogenized in a commercial food processor which was thoroughly cleaned between samples.

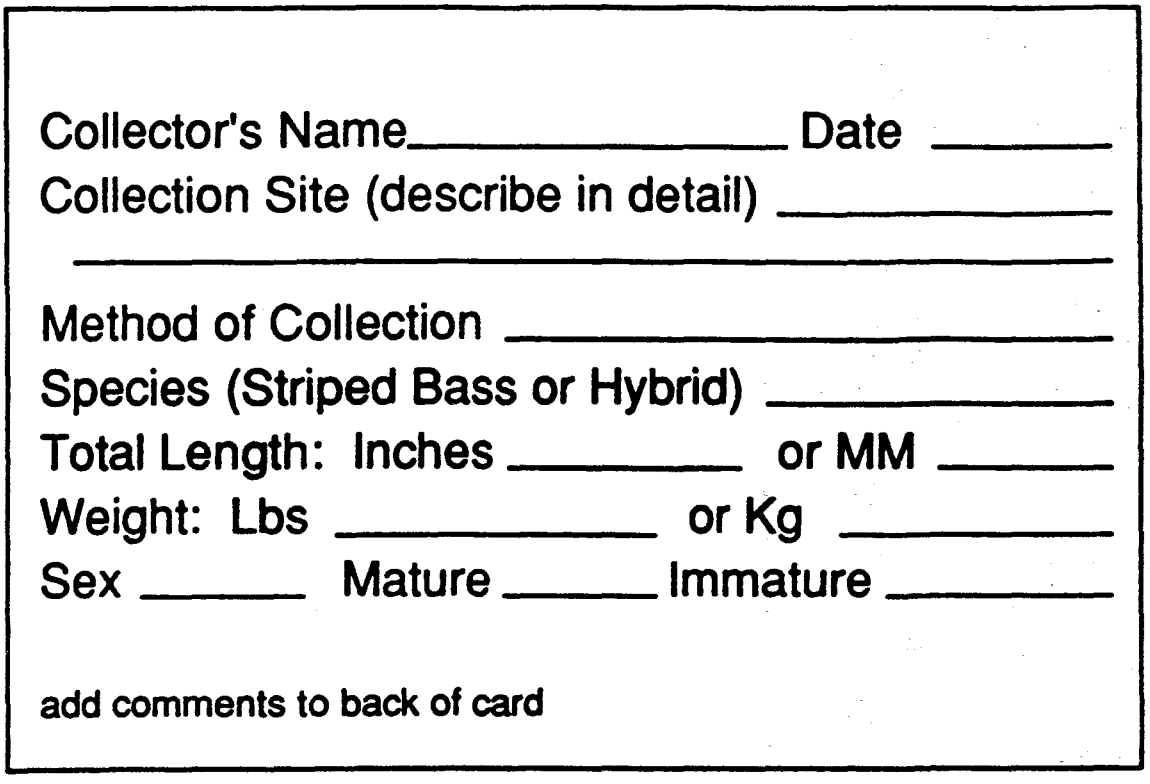

Figure 1. Collection card for the wild striped bass and wild hybrid striped bass fatty acid study.

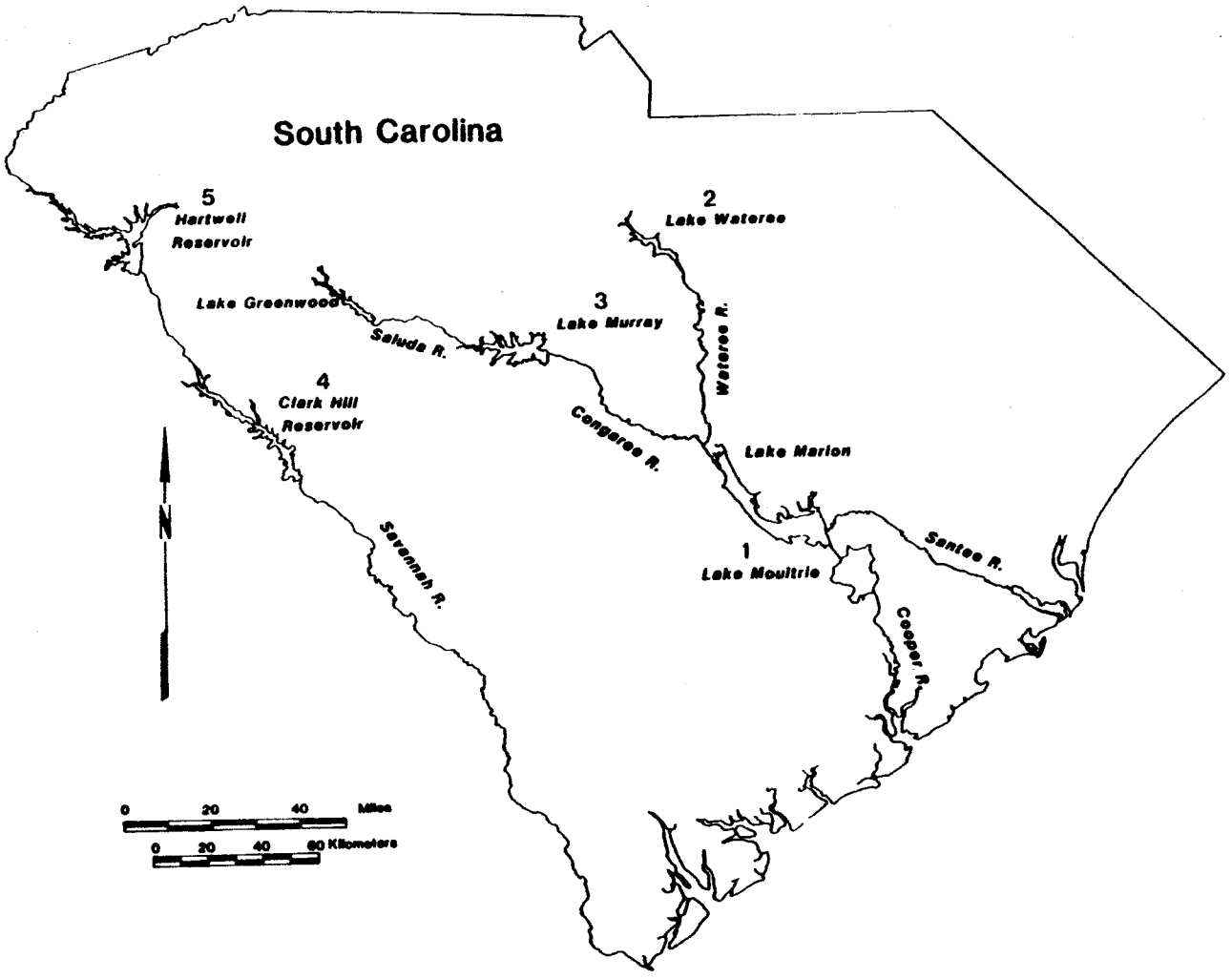

Figure 2. Wild fish collection sites for the fatty acid study in South Carolina.

Fatty Acid Analysis of Reference Fish and 'Test Samples'

Lipids were extracted from duplicate 5 g portions of each individual homogenized sample or composite sample using a chloroform-methanol extraction method (Folch et al. 1957).

Fatty acid methyl esters were prepared by the method of Metcalf et al., (1966). The esters were analyzed by gas-liquid chromatography utilizing a Hewlett-Packard (H-P) 5890 gas chromatograph equipped with flame ionization detector and H-P 3396A electronic integrator. The integrator was interfaced with a personal computer to facilitate direct transfer of chromatographic data to the computer for processing, storage and statistical analyses. Separation of FAME was achieved on a wall-coated open tubular (WCOT) flexible-fused silica 30m x 0.25mm ID DB225 column (J&W Scientific). Helium, the carrier gas, was used at a flow rate of 1.5 ml/min. Nitrogen was used as the make-up gas. Analytical runs were temperature programmed from 170° to 225°C @ 1°/min. Injections were in the split mode with a split ratio of 1:50.

Fatty acids were identified by comparison of their equivalent chain length values, calculated from isothermal runs, with those of primary and secondary standards (Jamieson 1970) and by gas chromatography/mass spectrophotometry (GC/MS) of the methyl esters.

RESULTS AND DISCUSSION

Wild Fish

All wild fish had linoleic acid (18:2n6) concentrations of less than 5% (Figure 3). Differences in linolenic acid (18:3n3), arachidonic acid (20:4n6) and docosahexaenoic acid (22:6n3) were evident among fish collected from different lakes.

Fatty acid composition of fish can be affected by diet, water temperature, salinity, seasonal variation, sexual maturity, physiological condition, etc. (Stansby 1981). Fish collected from Lakes Hartwell, Thurmond (Clarks Hill) and Murray contained higher concentrations of docosahexaenoic acid (22:6n3) than did fish collected from Lakes Wateree and Moultrie. Lakes Hartwell, Thurmond and Murray are also deeper and colder than Lakes Wateree and Moultrie (Miller White 1988, personal communication). A general trend exists toward higher content of long chain polyunsaturated fatty acids at lower water temperatures. The greater degree of unsaturation may allow for increased flexibility of cellular membranes at lower temperatures (Halver 1980).

Fish collected from Lake Wateree had the highest linolenic acid (18:3n3) concentration. Lake Wateree is the most productive of all the lakes and has the largest biomass of plankton and largest standing crop of fish. It is highly productive since it receives high phosphate run-off from the city of Charlotte, NC. (Val Nash 1988, personal communication). Algae are an excellent source of linolenic acid (18:3n3) (Ackman et al. 1964). The higher linolenic acid (18:3n3) concentrations in fish collected from Lake Wateree may be due to food chain transfer from smaller fish feeding on the algae to the bass feeding on the smaller fish.

Currently we are analyzing our fatty acid data to determine what effect sex, sexual maturity, season and collection site have on the fatty acid composition of these wild fish.

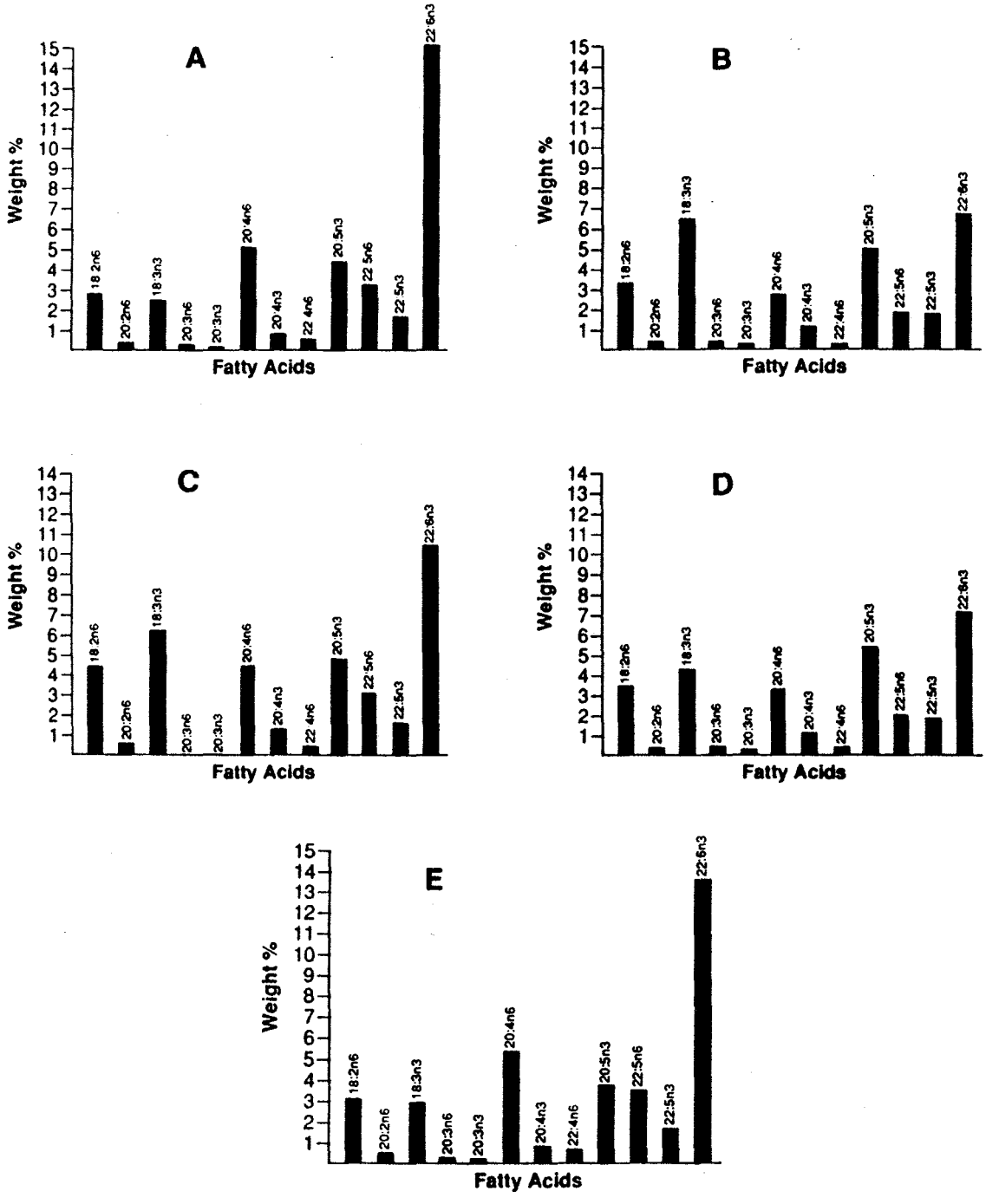

Figure 3. Percentages of selected fatty acids typical for wild fish collected from lakes A. Hartwell, B. Wateree, C. Murray, D. Moultrie and E. Thurmond (Nov. 1988). (Fish ranged in weight from approximately 450-850g).

Cultured versus Wild Fish

The fatty acid composition of cultured hybrid striped bass reflected the fatty acids contained in the diet (Figure 4).

In Figure 5 the concentrations of selected long chain polyunsaturated fatty acids contained in cultured hybrid striped bass and wild striped bass, are compared with those of 'Test Sample C' and 'Test Sample B'. The linoleic acid concentration is several times higher in the cultured hybrid striped bass reference (11%) and in Sample C (13%) than in the wild striped reference (3%) or in Sample B (3%). 'Samples A, D, E, F and G' were also found to have low concentrations of linoleic acid (1.2 - 4.9%) (Table 1). Based on linoleic acid concentrations Sample C was identified as a cultured fish and the other six samples as wild fish. These findings were confirmed by the Law Enforcement Official who collected the samples. Our research shows that cultured hybrid bass contain higher concentrations of linoleic acid than do wild striped bass or wild hybrid striped bass. This difference, due to high concentrations of linoleic acid in manufactured fish feeds, can be used as a tool to distinguish cultured fish from wild fish.

Table 1. Selected Fatty Acids of 'Test Samples A, D, E, F and G' . Data are expressed as weight percent of total fatty acids.

Fatty Acids	Test Sample A	Test Sample D	Test Sample E	Test Sample F	Test Sample G
18:2n6[a]	4.9	1.2	2.6	2.8	3.2
20:2n6	0.8	0.3	0.2	0.3	0.6
18:3n3	4.0	0.7	0.7	1.7	3.1
20:3n6	0.5	0.3	0.2	0.7	0.3
20:3n3	0.3	0.1	0.1	0.2	0.3
20:4n6	5.9	7.1	17.0	12.9	6.3
20:4n3	0.9	0.5	0.5	0.5	0.9
22:4n6	0.7	0.5	1.0	1.7	0.6
20:5n3	3.7	9.8	5.1	4.6	4.7
22:5n6	4.5	1.3	2.0	6.3	4.2
22:5n3	2.6	3.3	4.4	3.1	1.8
22:6n3	12.4	17.6	21.5	17.6	17.2

[a]linoleic acid

Although found in natural foods, linoleic acid is especially high in commercial fish feeds since soybean meal is often used as a major ingredient and soybean oil contains approximately 54% linoleic acid. Such high concentrations are not found in the wild fish's natural diet.

We also speculated that 'Test Sample C' had at one time fed on natural foods. This assumption was based on the higher concentrations of linolenic acid (18:3n3) and arachidonic acid (20:4n6) in the flesh of Sample C (Figure 5). Such concentrations are present in wild fish but not in cultured fish fed this commercial diet.

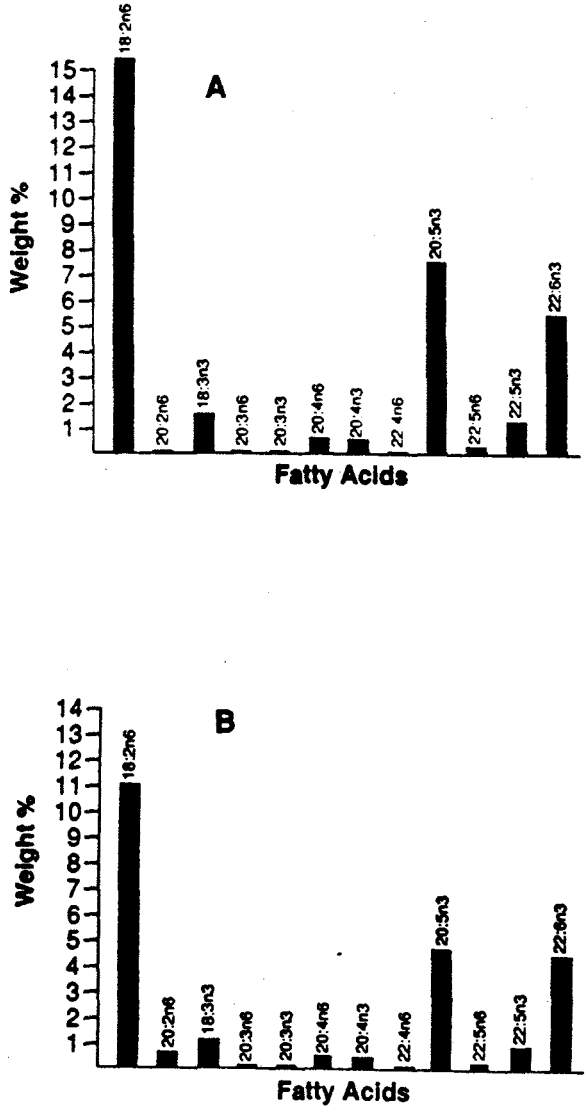

Figure 4. Representative fatty acids in A. a commercial diet and B. cultured hybrid striped bass (3 fish composite), respectively. (Fish ranged in weight from approximately 818-903g).

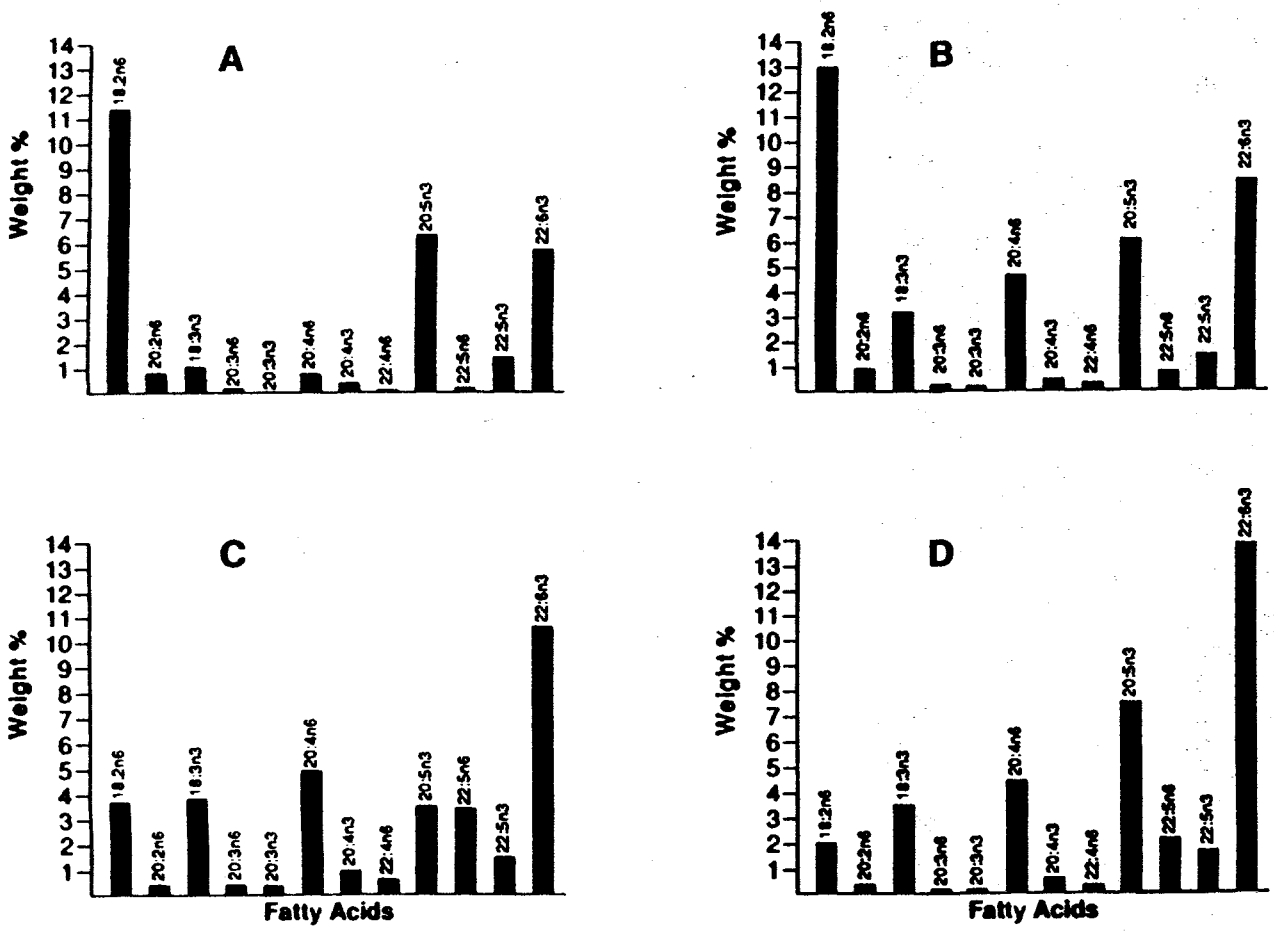

Figure 5. Comparison of selected fatty acids in cultured hybrid striped bass and wild striped bass.
- A. Cultured hybrid striped bass
- B. Unknown sample C - identified as cultured hybrid striped bass
- C. Wild male striped bass - reference standard
- D. Unknown sample D - identified as wild striped bass.

CONCLUSIONS

Wild Fish

All wild fish contained low concentrations of linoleic acid (18:2n6). Differences in fatty acid composition existed in fish collected from different lakes. Additional research may result in the ability to determine the specific lake from which the wild fish were collected using differences in fatty acid profiles.

Wild/Cultured Differentiation

The fatty acid composition of fish reflected the fatty acids found in their diet. Based on differences in fatty acid compositions, particularly the linoleic acid (18:2n6) concentration, a successful identification was made for the fillets that came from a cultured (farmed) fish (Sample C) as well as those that came from wild fishes (Samples A, B, D, E, F, and G).

Our current research indicates that in addition to linoleic acid, differences in the concentrations of several other long chain polyunsaturated fatty acids such as linolenic acid (18:3n3), arachidonic acid (20:4n6), docosapentaenoic acid (22:5n6) and docosahexaenoic acid (22:6n3) could also be used to help distinguish wild from cultured fish.

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